RED CELL MORPHOMETRICS AND VOLUME IN THE CICHLID TELEOST TILAPIA MOSSAMBICA, USING A CHROMIUM-51 LABELING METHOD[1, 2]

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Investigations of blood and blood cell volumes are limited in fishes, but a cross-section of interpretation of blood cell morphology is offered in the reports of Downey (1909), Jordan and Speidel (1930), Catton (1951) and Weinreb (1963). Blood volume studies have been hampered because the methods used to measure the mass of red cells were inadequate for application to fish. Exsanguination has been frequently attempted with limited success (Ronald, MacNab, Stewart and Beaton, 1964). Derrickson and Amberson (1934) used a perfusion technique on the dogfish, replacing blood with isotonic saline. Indirect dye dilution using Evans Blue was applied by Thorson (1961) on several marine and fresh-water species and by Smith and Bell (1964) on pink and sockeye salmon. Ronald *et al.* (1964) have applied fluorescein dye dilution on the Atlantic cod and Conte, Wagner and Harris (1963) used ^{51}Cr, Evans Blue and human serum albumin-^{131}I for blood studies on steelhead trout. The purposes of this investigation were to describe the formed elements and derive the red cell and total blood volumes of *Tilapia mossambica* and to determine the usefulness of ^{51}Cr as a labeling material in this fish.

Methods and Materials

The experimental species, *T. mossambica*, was collected from an estuary of the Enchanted Lakes located near Kailua on the windward side of Oahu, Hawaii. The size range was from 150 to 250 mm. (fork length) with weights ranging from 100 to 250 g. Females were of much greater relative abundance than males. The surface temperature of the estuary averaged 26° C. and the salinity varied from 0 to 25 ppt. Captured fish were held in 55-gallon fiberglass aquaria containing flowing fresh water with a steady temperature of 23.3° C.

Experimental animals were anesthesized with MS 222 (Tricaine methanesulfonate) in a 1:10,000 weight-to-volume solution to a deep stage III level, which is characterized by an absence of swimming movements, respiratory activity or response to external stimulation (Klontz, 1964). They were then placed in a restraining device to irrigate the gills with the MS 222 solution using a pump recycling system. The equipment was similar to that used by Smith and Bell (1964) at the Nanaimo, British Columbia, Station of the Fisheries Research

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Board of Canada. Because of the unreliability of heart puncture and injection, a technique for cannulation of the ventral aorta was utilized using a variation of a dorsal aorta cannulation employed by Schiffman (1959) and Smith and Bell (1964). The cannula was polyethylene tubing with an inside diameter of 0.025 inch. The tubing was placed over the cut end of a 22-gauge regular bevel needle and filled with a solution of 1% NaCl, 1.2% dextrose and 220 units of heparin per milliliter. The needle was inserted into the skin 4 or 5 mm. to either side of the median sagittal axis of the fish and directly above the ventral aorta. A suitable vessel puncture site was easily selected as the needle and vessel were visible through the membrane of the isthmus. As an attempt to maintain the fish in a constant but an admittedly abnormal environment, the animal was completely immersed in a small blackened aquarium which was supplied with flowing fresh water.

While no tissue erosion was noted around the sutures or puncture sites, hemorrhages developed at the puncture site one to two days after cannulation and after three days, successful sampling could no longer be carried out. It was possible to remove 30 to 50% of the total volume of blood in *Tilapia* without aspiration and no mortalities occurred following repeated injection and blood removal, but it often took the fish 3 or 4 hours to recover to pre-anesthesia levels of activity and appearance.

Smears of fresh, unheparinized blood were used for descriptive study. Smears were fixed in absolute methanol and flooded with Giemsa stain buffered with Sorensen's phosphate buffer to pH 6.8. Red cell dimensions of fixed cells were measured by use of an eyepiece micrometer. Thickness measurements were obtained from living cells in suspension. No differential white cell counts were made. Hematocrits were determined using the micromethod of Snieszko (1960). They were taken from all ventral aorta cannulated fish, serving as controls for blood volume values and were also taken in some fish by severing the tail and exposing the caudal vessels. In each microhematocrit determination three tubes were filled consecutively.

Chromium-51 was supplied as $Na_2{}^{51}CrO_4$ with a specific activity of 20 mc. per milligram. The ^{51}Cr activity of liquid samples, in microhematocrit capillary tubes calibrated to a volume of 0.04 ml., was measured with a crystal scintillation detector. At least two five-minute counts were made on each sample. For labeling, 10 μc. of ^{51}Cr were added to each milliliter of heparinized whole blood in a silicon-coated vial. After this mixture was incubated at room temperature (24° C.) for 75 to 90 minutes, with shaking every 5 minutes, 4 mg. per 10 μc. ^{51}Cr of ascorbic acid were added to reduce free ^{51}Cr. The optimum incubation time was determined from the time labeling activity reached a maximum. At 1 to 10, 20, 30, 45, 50, 60, 75, and 90 minutes after adding ^{51}Cr to a vial of unlabeled blood, 0.01-ml. subsamples of blood were withdrawn and the red cell ^{51}Cr activity was measured. The labeling was inhibited by the addition of an aqueous solution of 0.5 mg. ascorbic acid or by washing the subsample with saline to remove free ^{51}Cr.

The total volume of red cells was calculated from a dilution formula described by Squibb & Co. (1959). About 0.5 to 0.9 ml. of ^{51}Cr-labeled whole blood was injected into the cannula of the donor fish with 0.1 ml. retained as a

TABLE I

The formed elements of T. mossambica blood in circulation

Cell type	Dimensions (microns)					Cell:nuc. ratios (by length)
	Whole cell			Nucleus		
	Length	Width	Thickness	Length	Width	
Small lymphocyte	7.5*		—	6.3*		1.2
Large lymphocyte	12.4*		—	5.8*		2.1
Macrophage	12.9*		—	7.9*		1.6
Thrombocyte	9.1	3.7	—	7.0	3.3	1.3
Erythrocyte**	11 ± 0.7	7.5 ± 0.1	3.0	5.0	3.4	2.2

* Recorded as the greatest dimension through the central axis.
** Numbers per mm.^{3} = 1,549,000 ± 73,000 SD.

reference standard. Serial sampling up to 24 hours post-injection was accomplished by filling capillary tubes directly from the tip of the cannula. Total blood volume was found by dividing the total sample volume of red cells by the respective sample hematocrit and computing an average for all samples.

RESULTS AND DISCUSSION

For comparative purposes the morphological categories of *Tilapia* blood cells were similar to those described by Catton (1951) and Klontz *et al.* (1964),

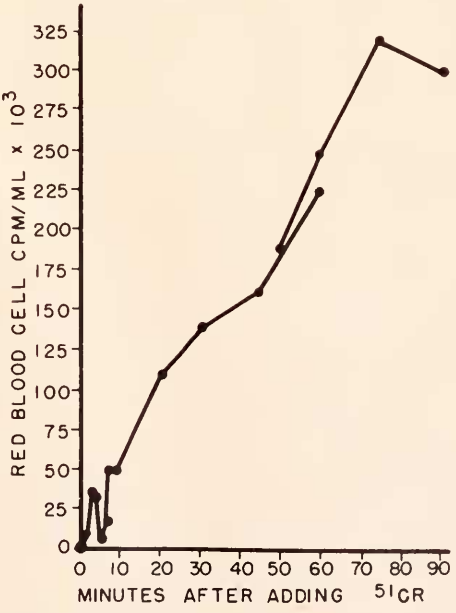

FIGURE 1. Relationship of *in vitro* ^{51}Cr accumulation by *Tilapia mossambica* RBCs as a function of incubation time.

except cells of the granulocytic series were absent in both living and stained preparations. Morphometric and enumeration data are presented in Table I. The average hematocrits were $25.8 \pm 4.6\%$ ($N = 29$) for caudal vessel samples and $23.0 \pm 3.8\%$ ($N = 9$) for ventral aorta samples obtained prior to blood volume determinations. The nearly 3% difference between the two mean hematocrits was not significant (analysis of variance test; $F = 2.81$, d.f. 1, 36), and probably was a reflection of differences in sampling. Sample hematocrits from ventral aorta cannulations, used for total blood volume determinations, presented a lower average ($20.5 \pm 4.1\%$, $N = 7$) than presample hematocrits. This was a result of the decrease in sample size and not of blood loss. There was also a tendency for hematocrits (caudal vessel samples) to decrease with increasing fish weights. For a wide weight range, this may be correlated with activity or oxygen consumption.

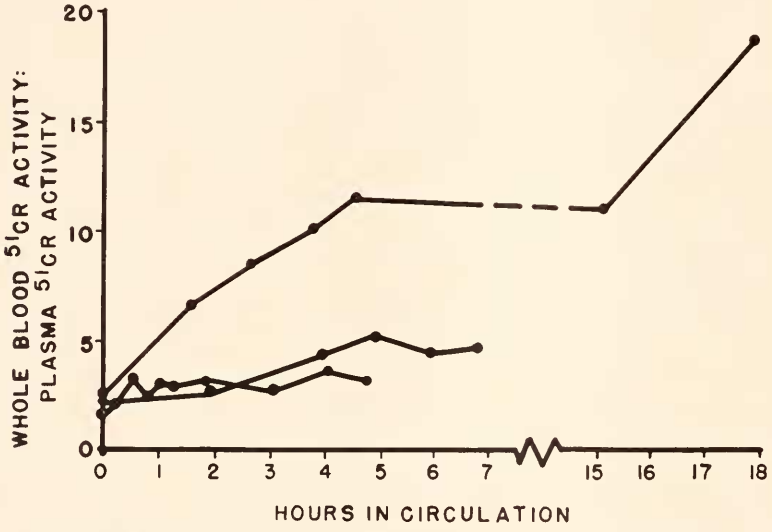

FIGURE 2. Whole blood ^{51}Cr activity:plasma ^{51}Cr activity ratios in three *Tilapia* versus the time of circulation.

The ^{51}Cr uptake curve (Fig. 1) is nearly linear with activity maximizing at 75 to 90 minutes after the addition of ^{51}Cr, although uptake is still rapid at 75 minutes. The dose-dependent effects or possible toxic levels of the ^{51}Cr were not tested. Plasma or unlabeled ^{51}Cr activity levels in *Tilapia* decreased to near background levels 4 to 19 hours after ^{51}Cr infusion in the circulatory system. Initially a large quantity of the radioactivity present in the injection medium does not appear in the red cells. There is a steady decline of plasma activity levels after injection, however, indicating that plasma ^{51}Cr is being rapidly removed from circulation and stored or excreted or both. In all fish samples (serial and single samples from 8 fish) the whole blood activity:plasma activity ratios in the injection medium range from 1.5 to 3.0. After 15 to 19 hours of circulation the values range from 10.2 to 18.8 (Fig. 2). Serial sampling indicates a period

of rapid mixing of injected labeled red cells in circulation during the first 10 to 60 minutes, followed by a long period of slow diffusion where fairly constant total red cell volumes or red cell ^{51}Cr activity levels were obtained. Nevertheless, the picture of dilution and circulation in the restrained or confined fish may not be an actual representation of what occurs while it is free-swimming. Since body movements probably aid peripheral circulation, the weak contractions of the heart while the fish is under anesthesia may supply only a limited flow of blood to body elements other than the head, gills, and viscera.

The mean value for the total blood volume was 3.17 ± 0.48 SD ml./100 g. body weight (range = 2.71 to 4.50). The mean value for the total volume of red cells was 0.65 ± 0.14 SD ml./100 g. body weight (range = 0.43 to 0.84, $N = 7$). A degree of correlation was found among blood volume against weight in seven fish which had moderately constant circulation times (Fig. 3). While the relationship was not significant at the 5% level, there was a definite inverse trend of volume on weight within the weight range tested. This indicates that blood volume in *Tilapia* is not directly proportional with weight as was found in the cod

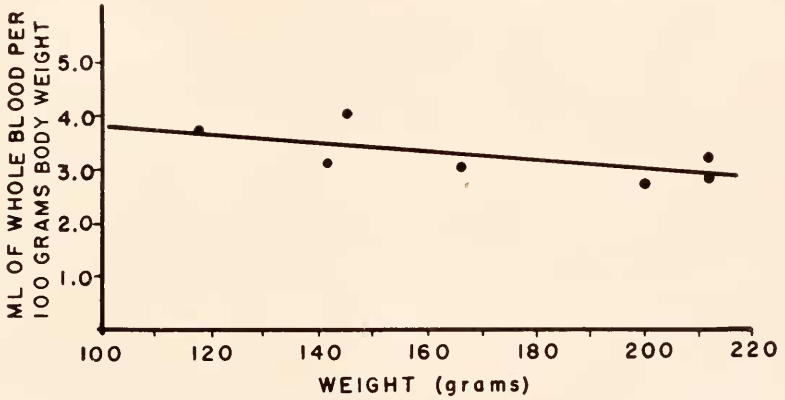

FIGURE 3. Relation of whole blood volume as a percentage of weight (ml. blood/100 g.) on total body weight in *Tilapia mossambica*. Circulation times after the injection of ^{51}Cr labeled RBCs were two to four hours.

(Ronald *et al.*, 1964) but decreases slightly with increasing weight. The total blood volume of *Tilapia* is consistent with those determinations made for other teleosts (Thorson, 1961; Conte *et al.*, 1963; Ronald *et al.*, 1964).

SUMMARY

1. Erythrocytes, small and large lymphocytes, macrophages and thrombocytes were identified. No granulocytes were found in stained or living preparations. Hematocrits averaged $25.8 \pm 4.6\%$ for caudal vessel samples and $23.0 \pm 3.8\%$ for ventral aorta samples. Red cell counts were $1{,}549{,}000 \pm 73{,}000$ SD per mm^3. Red cells were 11 ± 0.7 μ long, 7.5 ± 0.1 μ wide and 3 μ thick.

2. A technique is described for cannulation of the ventral aorta to allow repetitive sampling or injection while the fish is restrained or confined.

3. The labeling of erythrocytes with ^{51}Cr followed a linear curve with maximum cell incorporation occurring at approximately 90 minutes post-labeling.

4. A long period of slow mixing was noted for injected ^{51}Cr-RBCs after an initial dilution phase of 10 to 60 minutes. The total average total volume of red cells obtained for *Tilapia* as determined by ^{51}Cr dilution was 0.65 ± 0.14 ml./100 g. body weight and the mean total blood volume was 3.17 ± 0.48 ml./100 g. body weight. There was an allometric relationship suggested between TBV as a percentage of body weight and weight. Higher proportional volumes were associated with lighter animals.

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